Nutritional quality of eggs from hens fed distillers dried grains with solubles

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ABSTRACT A feeding trial was conducted with laying hens where either 10% or 20% regular-fat distiller's dried grains with solubles (R-DDGS) or low-fat DDGS (L-DDGS) were incorporated into the feed. Production parameters and the effect of DDGS on egg nutritional quality, focusing on yolk lipids, were evaluated. Neither R-DDGS nor L-DDGS at up to 20% of laying hen feeds had a statistically significant impact on hen weight gain, egg production, feed intake, feed efficiency, egg mass, or egg weight. Specific gravity was slightly lower for eggs from hens fed 10% R-DDGS or 20% L-DDGS. Eggs from layers fed DDGS had enhanced levels of tocopherols, tocotrienols, and xanthophylls in the yolk, as well as also increased yolk yellow and red color. Eggs from L-DDGS diet had higher tocopherol content, but eggs from R-DDGS diets had higher xanthophylls. Fatty acid composition in eggs was slightly altered by DDGS, but the ratio of saturated to unsaturated fatty acids was very similar. Feeding DDGS to layer hens had no effect on lecithin or cholesterol content of the eggs. Thus, inclusion of DDGS in the diet of laying hens resulted in increases of several beneficial lipophilic nutrients in egg yolks with no apparent detrimental effects.

Key words: distiller's dried grains with solubles, laying hens, egg production, egg color, nutritional quality

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INTRODUCTION

Distiller's dried grains with solubles (**DDGS**) are a co-product of the fuel ethanol industry: as the starch part of the grain (usually corn) is fermented into ethanol, the non-fermentable residue is left behind. DDGS have been used as feed for several decades. DDGS production has increased 6-fold in the past decade, as the dry-grind ethanol industry in the United States has expanded. For example in the 2011 to 2012 year, production of DDGS was estimated at around 40 million tons, about one-third of which was exported, and the remainder sold domestically (Wisner, 2013). While demand for DDGS as feed has increased, new markets have also been sought.

Due to starch fermentation, the non-starch components of corn including fiber, lipids, minerals, and protein, are concentrated (∼3-fold) in DDGS (Liu, 2012). The oil in corn is concentrated from 2.5 to 3% to 8 to 13% in DDGS, which has made it profitable for many ethanol processors to separate some of the oil, typically via centrifugation at the back end of processing. The oil can be sold as an additional valuable coproduct, and the protein and fiber in the resulting low-fat DDGS has even higher protein and fiber content. Studies have shown that many of corn's natural lipophilic phytochemicals, including phytosterols, tocopherols (Vitamin E), and carotenoids, are also enriched in DDGS (Winkler et al., 2007; Moreau et al., 2010; Moreau et al, 2011). It is estimated that over 90% of Americans do not consume enough Vitamin E in the diet to meet the estimated average requirements (Moshfegh et al., 2005). In addition, there is a growing body of evidence, as summarized by Aggarwal et al. (2010) that tocotrienols, which are members of the Vitamin E family, have anti-cancer, cholesterol lowering, and neuroprotective action. Lutein and zeaxanthin are antioxidant carotenoids that may help protect eyes from the development of cataracts and age-related macular degeneration (Basu et al., 2001; Abdel-Aal et al., 2013), however the typical United States diet does not contain the levels shown to be related to reduced risk (Seddon et al., 1994). In addition, carotenoids are often added as a supplement to the feed of broilers and laying hens in order to enhance skin color and egg yolk color, respectively. Since DDGS is enriched in many of these components, it is of interest to determine if the incorporation of DDGS in laying hen diets might increase their concentrations in egg yolks, thus enhancing their overall nutritional quality.

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Early studies show that DDGS can be fed to laying hens up to 20% inclusion rates without negatively affecting egg production or other production parameters (Robertson et al., 2005, reviewed by Batal and Bregendahl, 2012). Largely, the studies that address the utilization of DDGS as feed, have mainly focused on the effect of DDGS as a feed component on animal growth and performance, e.g., broiler growth or layer egg production, while only a few have looked at the effect of feed DDGS on the nutritional quality of eggs (Jiang et al., 2013; Sun et al. 2013). We are only aware of one study that has investigated the effects of low-fat DDGS on production factors (Purdum et al., 2014); this study found that even without adjusting the ME or kilocalorie per day, the lower fat DDGS had no significant effect on production parameters for young Bovan laying hens over a thirteen-week period.

The objective of this study was to determine the effect of inclusion of regular and low-fat DDGS in laying hens diet on egg production and nutritional quality, including egg yolk color and lipid composition. Since DDGS has high concentrations of lipophilic phytochemicals, such as lutein, zeaxanthin, tocopherols, and tocotrienols, our hypothesis was that the inclusion of DDGS in the diet would result in higher concentrations of these phytochemicals in the egg yolk. Egg yolk cholesterol was also analyzed because of its human nutritional importance, and phospholipids were analyzed because of their functional importance when eggs are used as food ingredients.

MATERIALS AND METHODS

Experimental Materials

Regular (13.3% fat) and low-fat (7.4% fat) DDGS were obtained from two different ethanol producers in IL. These samples were sealed and stored in a −20°C freezer until ready to mix with the feed as noted below. The proximate composition and lipid composition of the DDGS samples are shown in Table 1, the essential amino acid profile is shown in Supplementary Table S1.

Chemicals

Tocopherols [97 to 98% pure, as determined by gas chromatography (**GAS**)] were purchased from Matreya (Pleasant Gap, PA). Tocotrienols (∼95.5% pure, as determined by GC) were from ChromaDex (Irvine, CA). Lutein (97% pure, TLC), zeaxanthin (97% pure, TLC), and β-cryptoxanthin (97% pure TLC) were purchased from Indofine (Irvine, CA). 5α-cholestane (98% pure) was purchased from Sigma (St. Louis, MO). All other chemicals and organic solvents were either American Chemical Society (**ACS**) grade or HPLC grade and were purchased from either Sigma or Fisher Scientific (Fair Lawn, NJ).

Table 1. Proximate analysis and lipid compositions of the R-DDGS and L-DDGS added to hen diets.[1]

	R-DDGS	L-DDGS
(wet weight basis)		
Moisture	11.2	10.1
Protein	30.7	30.6
Fat	13.3	7.4
Fiber	7.3	6.5
Ash	4.9	4.9
Fatty acids (area %)		
C16:0	11.33	11.86
C16:1	0.14	0.13
C18:0	1.73	1.93
C18:1	27.0	27.4
C18:2	57.7	56.3
C18:3	1.5	1.6
Other Lipids (mg/kg)		
α-Tocopherol	20.9	20.1
β-Tocopherol	0.45	0.37
γ-Tocopherol	76.0	38.3
δ-Tocopherol	1.4	0.9
α-Tocotrienol	10.9	8.8
γ-Tocotrienol	17.4	9.0
δ-Tocotrienol	1.4	0.3
Total (tocopherols + tocotrienols)	128.6	77.8
Lutein	15.7	39.3
Zeaxanthin	9.4	9.7
β-cryptoxanthin	3.3	3.4
Unknown	1.6	3.7
Total Xanthophylls	29.9	56.1

[1]Analyzed content does not include residual and soluble carbohydrates. Mass closure was not performed on the proximates.

Experimental Diets

Five diets were fed to 150 Hyline W-36 White Leghorn hens from 21 to 41 wk of age. Each feed group contained 30 hens, selected at random from the pool of 150. Experimental diets were standard corn and soybean meal diets with a control diet devoid of DDGS, diets containing 2 levels of regular (13.3% fat) DDGS (**R-DDGS**, 10% or 20%), and diets containing 2 levels of low-fat (7.4% fat) DDGS (**L-DDGS**, 10% or 20%), as shown in Table 2. Diets were also supplemented with poultry fat in order to meet breeder recommendations for TME. Diets were isonitrogenous, containing 18.5% calculated CP and 2,890 kcal/kg TME, and were also formulated to meet breeder recommendations for digestible amino acid and to have the same levels of digestible lysine, TSAA, and threonine among the diets. To prevent spoilage during storage, the diets were prepared in 2 batches approximately ten weeks apart; the first batch was fed from 21 to 33 weeks of age, and the second batch was fed from 34 to 41 weeks of age. A dietary sample was taken at the beginning of the study and analyzed for proximate composition, amino acid composition, minerals (Ca, P, and Fe), fatty acid composition, and lipophilic nutrients (tocopherols and xanthophylls).

Birds and Housing

One-hundred and fifty (150) Hyline W-36 White Leghorn pullets were reared according to the breeder's

Table 2. Experimental diet formulations and calculated and analyzed nutrient values (as fed basis).

Ingredient	Control	10% R-DDGS (13.3% oil)[1]	20% R-DDGS (13.3% oil)	10% L-DDGS (7.4% oil)	20% L-DDGS (7.4% oil)
Corn	49.6	45.7	41.8	45.1	40.6
Soybean meal	30.5	25.0	19.5	25.1	19.8
DDGS	-	10.0	20.0	10.0	20.0
Poultry fat	5.5	4.8	4.2	5.3	5.1
Limestone	10.8	11.0	11.1	11.0	11.1
Dicalcium phosphate	2.6	2.4	2.2	2.4	2.2
Salt	0.47	0.43	0.38	0.43	0.38
Mineral Premix[2]	0.09	0.09	0.09	0.09	0.09
Vitamin Premix[3]	0.03	0.03	0.03	0.03	0.03
DL-Methionine (99%)	0.27	0.27	0.26	0.26	0.25
L-Lysine, (79% Lysine)	0.06	0.17	0.30	0.16	0.27
L-threonine	0.04	0.07	0.11	0.06	0.09
Estimated content[4]					
TME	2,890	2,890	2,890	2,890	2,890
Total N (%)	2.96	2.96	2.96	2.96	2.96
Ca (%)	4.76	4.76	4.76	4.76	4.76
Available P (%)	0.60	0.60	0.60	0.60	0.60
Lysine (%)	0.96	0.96	0.96	0.96	0.96
Total TSAA (%)	0.80	0.80	0.80	0.80	0.80
Analyzed content[5] (%)					
Moisture	10	11	10	9.5	9.5
Total N	2.82	2.77	2.93	2.73	2.98
Crude Fat	7.8	8.8	8.8	8.7	9.1
Crude Fiber	2.2	2.8	3.1	2.4	3
Ash	19.3	15.1	16.3	18.8	17.3
Ca	5.44	5.56	5.43	5.13	5.00
P	1.07	1.07	1.02	0.97	1.01
Fe	0.47	0.41	0.40	0.45	0.40
K	0.95	1.08	1.01	0.94	0.97

[1]Analyzed essential amino acid analysis of diets are available in supplementary Table S1.

[2]The mineral premix provided (per kg diet): magnesium, 10 mg; manganese, 100 mg; zinc, 100 mg; copper, 10 mg; iodine, 0.7 μg; and selenium, 200 μg.

[3]The vitamin premix provided (per kg diet): vitamin A, 6,605 IU; vitamin E, 14.31 IU; cholecalciferol, 2,200 IU; menadione, 880 μg; vitamin B12, 9.3 μg; biotin, 33.0 μg; choline, 357 mg; folic acid, 1,100 μg; niacin, 33.0 mg; pantothenic acid, 8.81 mg; pyridoxine, 0.88 mg; riboflavin, 4.4 mg; thiamine, 1.1 mg; iron, 102.5 mg.

[4]Analyzed content of essential amino acids in the regular and low-fat DDGS samples and in the diets are available in supplementary Table S2.

[5]Analyzed content does not include residual and soluble carbohydrates. Mass closure was not performed on the proximates.

management guide. At 21 weeks of age, pullets were placed in a completely enclosed fan-ventilated building with wire cages and subjected to 16 hours of light and 8 dark (16L:8D) per day. Three cages of one laying hen/cage were considered an experimental unit or replication. Ten replications were randomly assigned to one of the five diets. Feed and water were given ad libitum throughout the 20 week period. All procedures concerning animal care and use were approved by the University of Georgia Committee on Laboratory Animal Care.

Proximate, and Mineral Analysis of DDGS and Feed Samples

Moisture was determined using NFTA method 2.2.25. Crude protein was measured by combustion by the AOAC method 990.3 (AOAC 2003). Crude fat in DDGS and feeds was determined by extraction in petroleum ether (AOAC 945.16), and crude fiber was determined by AOAC method 978.10.

Mineral Analysis. The DDGS and feeds were analyzed for calcium, phosphorous, potassium, and iron after microwave digestion in nitric acid followed by inductively coupled plasma (ICP, Perkin Elmer) using a modified AOAC method 968.08.

Laying Performance and Egg Quality

Hen day egg production was recorded daily and production percentage calculated weekly. Egg weights were recorded every five wk after all eggs were collected for that day. Egg mass was calculated by multiplying egg weight by egg production percentage for that week, divided by 100. Body weight and feed intake were measured every five wk and feed efficiency was calculated by dividing feed intake by egg mass. Hen mortality was monitored daily. Every four wk, one day egg production was used to determine egg specific gravity (range of salt solutions, from 1.060 to 1.095) and to measure egg yolk color using a Minolta colorimeter (CR300, Minolta Corp. Ramsey, NJ) which measures color by three axis values of L^*, representing black to white (0 = black, 100 = white); a^*, representing red and green (− = green,

+ = red); and b* representing yellow and blue (− = blue, + = yellow).

Egg Sampling

Eggs were collected at the end of the feeding trial (20 wk) for analysis of lipophilic nutrients and antioxidant activity. Eggs from each replicate were pooled. The egg whites were separated from the yolk, and the yolk was mixed before being evenly divided into three portions for analysis by the NCERC (minerals and amino acids – not reported in this study), the USDA-ARS (fatty acids and lipids), and Tufts University (antioxidant and FRAP).

Egg Yolk Lipid Extraction

Total lipids of 4 g egg yolk were extracted with 80 mL chloroform:methanol (2:1 v/v) according to Folch et al. (1957). After extraction, solvents were removed by rotary evaporation (Buchi R-215, New Castle, DE) and lipid weight was determined gravimetrically. The lipid extract was dissolved in a small amount of chloroform, and stored in capped amber vials at −80°C until the fatty acids and lipophilic nutrients could be analyzed.

Feed and DDGS Lipid Extraction

Lipids were extracted from DDGS and feeds using AOAC Official Method 2003.06 using a Soxtec extractor (Foss 2043, Eden Prairie, MN). Lipid fractions were redissolved in a small amount of chloroform, and stored in amber vials at −80°C until fatty acids and lipophilic nutrients could be analyzed.

Analysis of Fatty Acid and Lipids

Oil samples were converted to fatty acid methyl esters (**FAME**) using methanolic-KOH as described by Ichihara et al. (1996). Fatty acid compositions were determined by GC analysis on an Agilent 6890 GC (Palo Alto, CA) with a Supelco (Bellefonte, PA) SP-2380 capillary column (30 m × 0.25 mm ID × 0.20 μm film). Helium was used as a carrier gas with a flow rate of 1 mL/min, the injector was held at 220°C, with a 50:1 split ratio, the oven was held at 185°C, and the FID was held at 220°C. Commercial FAME standards GLC 15a (Nu-Chek Prep, Elysian, MN) and Supelco 37 component FAME Mix were used to identify peaks and verify GC performance.

Tocopherols and tocotrienols were analyzed by HPLC with a Varian (Agilent, Inc., Santa Clara, CA) Pro-Star pump, autosampler, and fluorescence detector. The mobile phase consisted of hexane:tetrahydrofuran (97:3 v/v) pumped at 2 mL/min. Oil samples were weighed and diluted in mobile phase, and tocopherols were separated using an Inertsil (GL Sciences, Inc., Torrance, CA), silica column [5 μm, 150 Å, 250 × 4.6 mm Internal Diameter (**ID**)]. The fluorescence detector was set with an excitation wavelength of 290 nm and emission wavelength of 330 nm. Data collection and integration were performed with Varian Star Chromatography Ver. 6.0. Tocopherol peaks were identified by retention time of pure standards and quantified using external standard curves at concentrations ranging from 0.5 μg/mL to 10 μg/mL. To verify performance of the system, a mixture containing α-, β-, γ-, and δ-tocopherol and tocotrienol standards was injected periodically to verify HPLC performance, and a soybean oil check sample was run each day to verify day-to-day coefficient of variation of ≤5%.

Xanthophylls were analyzed in the egg lipids by HPLC (Shimadzu LC20AT, Columbia, MD) equipped with a diode array detector (SPD-M20A) and an YMC (YMC America, Inc. Allentown, PA) carotenoid column (C30, 4.6 mm × 250 mm × 3 μm). The binary gradient program consisted of a linear gradient from 100% solvent A (methanol/methyl tert-butyl ether/water 81/15/4) to 70% solvent B (methanol/methyl tert-butyl ether/water 8.5/87.5/4) over 32 min, followed by a return to 100% solvent A in 5 min with a 5 min hold. Standard curves were developed using pure lutein, zeaxanthin, and β-cryptoxanthin standards ranging from 0.01 to 10 μg/mL. The hexane used for extraction of feed and DDGS lipids was too non-polar to efficiently extract slightly more polar carotenoids such as xanthophylls. Therefore, the procedure described by Kurilich and Juvik (1999) was used to extract the xanthophylls from the feed and DDGS samples prior to analysis of xanthophylls as described above.

Egg phospholipids were analyzed by HPLC using a method adapted from Becart et al. (1990). The Shimadzu HPLC system described above was used with an evaporative light scattering detector (ELSD, LTII). The HPLC column was a Lichrosorb Si60 (4.6 mm × 250 mm × 5 μm, Phenomenex, Torrance, CA). The ELSD was operated at 50°C, 3.0 bar nebulizing gas (ultra-pure nitrogen) and gain setting one. External standard curves using 99% pure egg phosphatidylcholine and egg phosphatidylethanolamine (Avanti Polar Lipids, Alabaster, AL) were used for quantitation.

Egg cholesterol was analyzed by GC after lipid saponification, derivatization, and quantification by internal standard with 5α-cholestane. The GC (Varian 3800, Agilent, Santa Clara, CA) was equipped with an FID, and a DB-5 (Agilent, 30 m × 0.25 mm × 0.25 μm) capillary column. Helium was used as a carrier gas, with a 1:50 injector split. Injector temperature was 270°C, and detector temperature was 290°C. The column oven initial temperature was 250°C for 0.5 min, increased at 10°C/min to 270°C and held for 27 min, then increased at 10°C/min to 280°C and held for 3.5 min.

Statistical Design and Analysis

Hen Performance and Egg Quality Analysis. A two-factor mixed model repeated measures ANOVA comparing feed intake, feed efficiency, % egg

production, egg mass, egg weight, egg specific gravity, and egg yolk L*, a*, and b* between the four diet treatments containing R-DDGS and L-DDGS along with a control was performed over four (egg weight, egg mass, feed intake, and feed efficiency), five (egg specific gravity and egg yolk L, a*, and b*) or 20 (egg production %) time periods. The experimental unit consisted of three cages, with one hen per cage, for a total of 30 cages (10 replicates) per dietary treatment. Mean values of the 3 hens per experimental unit were used. A Levene's homogeneity of variance test was performed to determine if any data transformations were needed. If a significant treatment main effect was obtained from an ANOVA, differences of least squares means were used as pairwise multiple comparison test at the $P < 0.05$ level. If a significant treatment × time interaction was obtained from an ANOVA, the SLICE option in SAS was used to examine treatment main effect differences at each time period. All analyses were performed on transformed data where necessary, but raw data means are presented for ease of interpretation. SAS v 9.2 (SAS, Cary, NC) was the software used for the analyses.

Egg Lipid Component Analysis. The effects of dietary treatment on egg lipid components were analyzed by one-way ANOVA. A Levene's homogeneity of variance test was performed and if unequal variances were found then Welch's ANOVA was used. Statistical significance was assumed at $P < 0.05$ to determine differences among the treatments. Where treatment differences were found, Tukey's HSD multicomparison test was performed to compare all treatment means. JMP 10.0 (SAS, Cary, NC) was used for the analyses.

RESULTS AND DISCUSSION

DDGS Component and Feed Lipid Composition

The proximate analysis of the two DDGS samples showed that the major difference in composition was in the crude fat content (Table 1), while protein remained very similar and fiber was slightly lower in L-DDGS. Recent fractionation and oil extraction technologies have allowed an even greater reduction of oil in DDGS to around 3%. In this study, soybean meal, corn, and poultry fat were adjusted to maintain equal TME. Batal and Dale (2006) analyzed TME_n and proximate composition of 17 DDGS samples ranging from 2.6 to 10.6% fat, and found that fat content was the best predictor for TME_n. However, the correlation coefficients for prediction models using the variables fat, fiber, protein, and ash ranged from 0.29 to 0.45, indicating that these equations could not accurately predict TME_n. A later study by Dale (2013) appeared to find a stronger correlation between fat content and TME_n, however, to the best of our knowledge, the detailed study has not yet been published. The lipid composition, including fatty acids, tocopherols, tocotrienols, and xanthophylls, of the oil extracted from each DDGS sample and feed treatment are shown in Tables 1 and 3, respectively. The R-DDGS and L-DDGS fatty acid compositions were similar to each other and to regular corn oil, with the major components being linoleic acid (C18:2), oleic acid (C18:1), and palmitic acid (C16:0). The feed fatty acid compositions were slightly higher in C16:0, C16:1 (palmitoleic), C18:0 (stearic acid), and C18:1, due to the additional use of poultry fat as a feed component. The addition of DDGS components in the diets resulted in slight decreases, compared to the control diet, in C16:0, C16:1, C18:0, and C18:1. However, these changes were subtle since corn oil and DDGS have similar fatty acid compositions, and the amount of poultry fat in the diets only varied slightly.

The oil extracted from L-DDGS was higher in α-tocopherol, α-tocotrienol, and overall tocopherol (**TOH**) content, but was slightly lower in γ-tocopherol, γ-tocotrienol, and δ-tocotrienol compared to the full fat DDGS. However, when calculated on as per g of DDGS, the L-DDGS was slightly lower in γ-tocopherol and γ-tocotrienol and overall TOH content because of its lower oil content. Incorporation of both R-DDGS and L-DDGS in the diets increased the content of all of the TOH components, with the exception of δ-tocopherol.

The major xanthophyll contributors in the feed are corn and DDGS. The DDGS components were analyzed by HPLC, and the major components were identified as lutein, zeaxanthin, and β-cryptoxanthin, while there were several other peaks which were not identified. The major unidentified peak was similar in the absorbance spectrum to lutein, so the lutein standard curve was used for its tentative quantitation. L-DDGS had higher content of lutein and the unknown xanthophyll, compared to the R-DDGS, but the two had similar zeaxanthin and β-cryptoxanthin content. However, analysis of the final feed components did not yield much higher contents of xanthophylls compared to the control diet, except for the diets with 10 and 20% R-DDGS. The lack of increase in xanthophylls is likely due to the fact that regular corn, which itself has 27 to 77 μg/g xanthophylls (Weber, 1987), was a larger component of the diet than either DDGS component, and its content was adjusted within each diet, along with the soybean meal.

Laying Performance

There was only dead hen throughout the study which occurred during the fourth week from the 20% L-DDGS treatment. There was no significant effect of diet, time, or diet by time interaction on hen body weight gain. Average hen body weight gain ranged from 0.15 to 0.20 kg/hen in the first four-week period, but there was negligible body weight gain thereafter for any treatment (data not shown). In a recent study, hen body weight gain was compromised by inclusion of 20% DDGS in the diet (Hassan and Al Aqil, 2015), but most studies have shown no impact of DDGS up to 20% as long as

Table 3. Lipid composition of experimental diets.

Lipid Component	Control	10% R-DDGS	20% R-DDGS	10% L-DDGS	20% L-DDGS
Fatty acid (% w/w)[1]					
C16:0	19.9	19.0	16.3	19.1	19.4
C16:1	4.3	3.8	2.8	4.1	4.1
C18:0	4.6	4.3	3.4	4.3	4.4
C18:1	35.6	34.5	32.3	35.0	35.0
C18:2	32.5	35.3	41.3	34.2	33.9
C18:3	1.9	1.8	1.8	1.9	1.7
Tocopherols (mg/kg diet)[2]					
α-T	5	6.1	10.4	6.8	7.3
α-T3	2.1	3	4.6	2.8	3.5
β-T	0.12	0.17	0.27	0.2	0.17
γ-T	16.8	19.1	32.4	20.2	19.4
γ-T3	1.6	3.1	5.4	2.6	3.3
δ-T	2.4	2	2.5	2.3	1.6
δ-T3	N.D.[3]	N.D.	N.D.	N.D.	N.D.
Total	28	33.3	55.7	34.9	35.6
Xanthophylls (mg/kg diet)					
Lutein	5.6	10.2	9.3	5.8	5.6
Zeaxanthin	2.6	4.1	3.1	3	2.9
B-cryptoxanthin	1.2	2	1.1	1.5	1.3
Unknown	0.3	0.6	0.6	0.4	0.4
Total	9.7	16.8	14.1	10.7	10.1

[1]Other fatty acid components, each <0.5%, include C14:0 and C20:0.
[2]Measurement by HPLC of free tocopherols only, exclusive of tocopheryl acetate from the vitamin mix, T = abbreviation for tocopherol, T3 = abbreviation for tocotrienol.
[3]Not detected.

the diets otherwise meet guidelines for ME and nutrient contents (Lumpkins et al., 2005; Roberson et al., 2005; Batal and Bregendahl, 2012).

No data transformations were needed for feed intake or feed efficiency. The transformation (egg mass)[4] stabilized the variance for egg mass and square root (% egg production) stabilized the variance for % egg production so that ANOVAs could be performed. There were no significant diet, time, or diet by time interactions for feed intake or feed efficiency (Supplementary Table S2a), but there were main effect diet treatment differences in % egg production ($P = 0.0286$). In this case, the effect of DDGS diet treatments was not significant compared to the control diet, which had on average 86.9% egg production, but hens with 20% R-DDGS or 10% L-DDGS had significantly higher egg production (87.7% and 88.4%, respectively) compared to hens fed either 10% R-DDGS or 20% L-DDGS (85.8 and 85.3%, respectively). Egg mass had a significant treatment x time interaction ($p = 0.0002$), so treatment differences in egg mass were examined at each time period (Supplementary Table S2b). Treatment differences in egg mass were found at time 2 ($P < 0.0001$), where egg mass from hens fed either 10% R-DDGS or 20% L-DDGS were slightly lower compared to the control diet and the other DDGS diets.

These results are in agreement with several reports (Lumpkins et al., 2005; Roberson et al., 2005; Masa'deh et al., 2011; Hassan and Al Aqil, 2015) showing that up to 20% regular DDGS can be incorporated into the diet of laying hens with no negative effect on feed intake, egg weight, or egg production. Most of these studies used regular DDGS while there are only a few published studies looking at the effect of low-fat DDGS on production factors. Purdum et al. (2014) found that when DDGS containing 5.2, 7.3, or 10.3% oil were incorporated at a level of 20% of a regular corn/soybean meal diet of 20 wk old Bovan White laying hens, they had no impact on any measured production factors over 13 weeks, even though the experimental diets were not adjusted to contain the same levels of metabolizable energy.

Table 4a. Main effect treatment differences of R-DDGS and L-DDGS on egg weight, specific gravity, and yolk L* and a* color.[1]

Treatment	Egg weight	Egg specific gravity	Egg L*	Yolk a*
Control	59.8	1.087[a]	58.54[a]	−4.29[d]
10% R-DDGS	59.9	1.083[c]	57.83[b]	−3.49[c]
20% R-DDGS	60.0	1.085[a,b]	56.72[c]	−2.19[a]
10% L-DDGS	59.2	1.085[b]	57.34[b]	−3.53[c]
20% L-DDGS	59.7	1.082[c]	56.63[c]	−2.71[b]
SEM (n = 10)	0.55	0.0007	0.256	0.105
P-value	0.62	<0.0001	<0.0001	<0.0001

[1]Measurement of egg weight, specific gravity, and egg yolk color were made on all available production day eggs every four weeks throughout the study. Results shown are the overall average by treatment, as well as the pooled standard error of the mean, and the *P*-value for the main effect of treatment. Treatment means within a column sharing the same letter are not significantly different based on Differences of Least Squares means at $P \leq 0.05$.

Egg Weight, Specific Gravity, and Color

No data transformations were needed for any of the five variables measured. There were no significant diet or diet by time effects on egg weight (Table 4a). However, significant main effect (diet) differences from ANOVAs were found for egg specific gravity, which was significantly lower for hens fed 10% R-DDGS, and for those fed either 10% or 20% L-DDGS, compared to the control diet. Roberson et al. (2005) also noted a slight

Table 4b. Interaction treatment differences of R-DDGS and L-DDGS on yolk b*color at each t time.[1]

Treatment	Time				
	1	2	3	4	5
Control	39.96^{c}	39.31^{b}	40.05^{d}	46.62^{a}	46.00^{b}
10% R-DDGS	43.13^{a,b}	43.68^{a}	44.56^{b,c}	47.27^{a}	48.57^{a}
20% R-DDGS	44.05^{a}	44.59^{a}	47.23^{a}	48.80^{a}	48.71^{a}
10% L-DDGS	41.67^{b,c}	42.65^{a}	42.65^{c}	47.05^{a}	47.42^{a,b}
20% L-DDGS	42.93^{a,b}	44.14^{a}	46.21^{a,b}	47.89^{a}	46.75^{a,b}
Slice p-value	0.0016	<0.0001	<0.0001	0.27	0.0479

[1]Measurements of egg yolk color were made on all available production day eggs every four weeks throughout the study. Treatment means for each time period (within a column) sharing the same letter are not significantly different based on Differences of Least Squares means at $P \leq 0.05$ using the SLICE option in SAS due to a significant ($P = 0.0286$) Treatment × time interaction from ANOVA.

decrease in egg specific gravity in eggs from hens fed DDGS, but it was only during one week period out of a nine-week study. The differences noted here may not be of practical importance since the overall differences between eggs with the highest and lowest average specific gravity were not as large as the increment between salt solutions, and in all cases, average egg specific gravity, an indicator of egg shell quality, were above 1.080.

Several studies have also shown that incorporating DDGS in the diet usually enhances egg yolk color (Roberson et al., 2005; Loar Ii et al., 2010; Masa'deh, 2011). Egg yolks from hens fed either form or level of DDGS had significantly lower L* values, representing black to white, compared to yolks from hens fed the control diet (Table 4a), meaning that they were significantly darker in color. In addition, the L* values decreased with increasing levels of DDGS in the diets. Egg yolks from hens fed DDGS also had significantly higher a* (red) color compared to the control, again in a dose dependent manner. There were no significant treatment differences in yolk b* (yellow) color, but there was a significant treatment*time interaction, so treatment differences were examined at each time period (Table 4b). Significant treatment differences in yolk b* color were found in time periods 1, 2, 3, and 5, but not 4. At all of these time periods, yolks from hens fed either level of R-DDGS had significantly higher b* color compared to the control. Yolks from hens fed 10% L-DDGGS had significantly higher b* compared to control yolks at time periods 2, and 3, and yolks from hens fed 20% L-DDGS had significantly higher b* compared to control yolks at time periods 1, 2, and 3.

Effect of R-DDGS and L-DDGS on Egg Yolk Lipid Composition

Lipid Content and Fatty Acid Composition. Since the egg yolks and whites were separated and egg yolks within each replicate were combined prior to analysis, the ratio of egg white weight to egg yolk weight is unknown. However, the total lipid content of egg yolks was not significantly affected by the diet (data not shown), and since egg weight was also not significantly affected by the diets it is valid to assume that the concentrations of lipid components in egg yolk lipids is proportional to their content in the whole eggs.

Several studies have shown that egg lipid fatty acid composition is affected by the fatty acid composition of dietary lipid components (Navarro et al., 1972; Fredriksson et al., 2006; Poureslami et al., 2012; Sun et al., 2013). The fatty acid composition of egg yolks in this study mainly reflected the composition of the diets and changes in some fatty acids were seen with increasing DDGS in the diets (Table 5). C16:1, originating from the poultry fat in the diet, decreased slightly, but significantly, in diets with either 10% or 20% R-DDGS in 20-week eggs, but was not significantly lower in eggs from hens fed diets with L-DDGS. Oleic acid (C18:1) in egg yolks was also lowered significantly by R- or L-DDGS in the diets, while linoleic acid (C18:2) increased significantly for diets with either R- or L-DDGS. Overall, the ratio of saturated fatty acids to unsaturated fatty acids (0.59) remained unchanged between all of the diets ($P = 0.6243$). Similar effects of DDGS on egg lipid fatty acid composition were recently reported by Jiang et al. (2013).

Tocopherol Content and Composition of Egg Yolk Lipids. After 20 weeks on the diets, α-tocopherol content was significantly higher in eggs from hens fed 20% L-DDGS (Table 5), and although the average α-tocopherol was higher in eggs from hens fed either level of R-DDGS, or 10% L-DDGS, the increase was not statistically significant due to high variation between replicates. Eggs from hens fed either 20% R- and 10% or 20% L-DDGS were significantly higher in α-tocotrienol at both 10 and 20 weeks. β-tocopherol levels were fairly low in all of the eggs, but were significantly higher in all DDGS diets. γ-Tocopherol and γ-tocotrienol contents were also higher from hens fed 20% R-DDGS as well as either 10% or 20% L-DDGS. Overall, while egg yolk lipids from diets with added DDGS all had higher (10% to 39%) average total tocopherols and tocotrienols, the increase in this total was only statistically significant for diets with 10% and 20% L-DDGS. Since the R-DDGS actually had higher total tocopherols and tocotrienols, one might speculate that the absorption of tocopherols by the hens may have been better from L-DDGS compared to regular DDGS, though no absorption efficiency data was collected for this study. However, the presence of high quantities of Vitamin E in the form of α-tocopheryl acetate provided in all diets through the Vitamin pre-mix likely interfered with the absorption of the other tocopherols and tocotrienols present in the diet. For example, Walker et al. (2014) found much higher amounts of tocotrienols in eggs from hens fed a diet supplemented with barley or palm oil tocotrienols. However, the diets did not contain any supplementary α-tocopheryl acetate, so the basal levels in the diets were much lower than in the present study.

Walker et al. (2012) demonstrated a dose-response relationship in α-tocopherol and tocotrienols content in

Table 5. Lipid components in egg yolks from layers fed experimental diets.

Lipid component	Control	10% R-DDGS	20% R-DDGS	10% L-DDGS	20% L-DDGS	SEM	*P*-value
Fatty acid composition[1]							
C16:0[2]	25.5	25.4	25.1	25.5	25.5	0.19	0.4
C16:1	2.71^{a}	2.46^{b}	2.08^{c}	2.54^{a,b}	2.49^{a,b}	0.06	<0.0001
C18:0	9.5	9.42	9.56	9.28	9.19	0.11	0.16
C18:1	45.7^{a}	43.5^{b,c}	42.1^{d}	44.4^{b}	42.3^{c,d}	0.31	<0.0001
C18:2	13.6^{c}	16.4^{b}	18.3^{a}	15.5^{b}	17.6^{a}	0.25	<0.0001
C18:3	0.44^{e}	0.45^{d}	0.47^{c}	0.45^{b}	0.58^{a}	0.04	0.0025
C22:0	2.10	2.10	2.20	2.10	2.10	0.04	0.82
Tocopherols and tocotrienols (μg/g oil)[3]							
α-T[1]	173.8^{b}	183.5^{a,b}	183.3^{a,b}	209.9^{a,b}	218.2^{a}	9.2	0.0032
α-T3	2.5^{c}	4.0^{b,c}	5.8^{a}	5.1^{a,b}	6.3^{a}	0.36	<0.0001
β-T	0.58^{c}	0.95^{b}	0.96^{b}	0.98^{b}	1.34^{a}	0.06	<0.0001
γ-T	46.0^{d}	57.2^{c,d}	72.2^{a,b}	67.0^{b,c}	85.1^{a}	3.5	<0.0001
γ-T3	0.13^{b}	0.23^{a,b}	0.34^{a}	0.26^{a}	0.32^{a}	0.03	<0.0001
δ-T	1.1^{a,b}	1.0^{a,b}	0.82^{b}	1.0^{a,b}	1.2^{a}	0.07	0.0056
Total	224.2^{c}	246.9^{b,c}	263.4^{a,b,c}	284.2^{a,b}	312.4^{a}	12.96	<0.0001
Xanthophylls μg/g oil							
Unknown	8.6^{d}	13.6^{b,c}	17.3^{a}	12.1^{c}	14.8^{a,b}	0.65	<0.0001
Lutein	80.0^{b}	110.1^{a}	123.1^{a}	87.4^{b}	91.1^{b}	4.2	<0.0001
Zeaxanthin	22.4^{c}	31.7 ^{a,b}	36.8^{a}	29.1^{b}	34.9^{a}	1.36	<0.0001
β-Cryptoxanthin	N.D.[4]	1.1^{b}	2.0^{a}	1.0^{b}	1.7^{a}	0.1	<0.0001
Total	111.1^{d}	156.4^{a,b}	179.1^{a}	129.7^{c,d}	142.6^{b,c}	6.22	<0.0001

$^{a–e}$Means in the same row with different superscripts are significantly different ($P < 0.05$) by Tukey's Honestly Significant Difference test.

[1]Other fatty acid components, each <0.5%, include C14:0 and C20:0.

[2]C16:0 = sixteen carbon fatty acid and zero double bonds.

[3]T = abbreviation for tocopherol, T3 = abbreviation for tocotrienol.

[4]Not detected.

egg yolks from hens fed diets that were supplemented with palm tocopherols and tocotrienols (the sum of tocopherols and tocotrienols supplemented beyond the basal diet ranged from 61.5 to 369 μg/g diet). Although the supplement was higher in tocotrienols (α−, β−, γ−, and δ− combined), α-tocopherol was present in the highest concentration in the egg yolk lipids and also had the highest transfer efficiency. α-Tocotrienol had the second highest transfer efficiency, despite that it was present at lower concentrations than γ-tocotrienol, which corresponds to our findings of more than 10-fold higher α-tocotrienol than γ-tocotrienol in egg yolk lipids, despite the fact that the concentration of these components in the feeds were about the same. Walker et al. (2012) found that tocopherols and tocotrienols levels in egg yolk lipids from hens fed supplemental palm tocotrienols increased in the first eight days of the study, and then steadily dropped to lower levels (although still above basal levels) for the rest of the feeding trial, which could possibly be explained as either feedback or competitive inhibition. A more comprehensive study of the tocopherols and tocotrienol levels in eggs from hens fed DDGS may be warranted to understand how feeding over time affects tocopherol and tocotrienol uptake.

Xanthophyll Content and Composition of Egg Yolk Lipids. Despite the fact that xanthophyll content in the experimental feeds were not dramatically increased compared to the control, total xanthophyll content was significantly higher in eggs from hens fed 10 to 20% R-DDGS or 20% L-DDGS. The dietary xanthophyll contents correlated well with the color changes measured in the egg yolks, including darker color (decreased L*-value) with increased yellow (a*-value) and red (b*-value) hues. In egg yolk lipids from the R-DDGS diets, significant increases were seen in lutein, zeaxanthin, β-cryptoxanthin, and the unknown xanthophyll. However, in egg yolk lipids from the L-DDGS diets, there was a significant increase in zeaxanthin, β-cryptoxanthin, and for the unknown xanthophyll, but no significant increase in lutein. Increases in xanthophyll content were consistent with several other studies confirming that carotenoid content and composition of egg yolk lipids can be influenced by dietary carotenoid sources (Hencken, 1992; Herber-McNeill and Van Elswyk, 1988; Frederiksson et al., 2006; Walker et al., 2012). However, the fact that there was not much difference in dietary carotenoid content between the control and DDGS containing diets indicates that there may be a better absorption or bioavailability of xanthophylls from DDGS compared to traditional corn feed. The cooking, fermentation, and drying processes that corn undergoes could release bound xanthophylls and improve their bioavailability in DDGS, but a more detailed study of the transfer efficiency would be needed in order to confirm this observation. Walker et al. (2012) found that with astaxanthin supplementation, as with tocotrienol supplementation, that concentrations increased in egg yolk lipids for the first eight days, and then slowly declined or leveled off over time. Thus, a further evaluation of carotenoid content in eggs over time, as affected by R-DDGS or L-DDGS treatments is

warranted to understand if there is a significant difference over time in carotenoid deposition as affected by the oil content and availability in DDGS.

Phospholipids, Cholesterol, and Antioxidant Activity. There was no significant effect of either R-DDGS or L-DDGS on phospholipid content and composition of phospholipids (data not shown), thus the normal production and utilization of choline and phospholipids were not affected by the addition of up to 20% R-DDGS or L-DDGS to the diet. Cholesterol content of the eggs was also not affected by the feed treatments. Since tocopherols, tocotrienols, and carotenoids have antioxidant activity, we measured ferric reducing antioxidant power (**FRAP**, Benzie and Strain, 1996) of the regular and low-fat DDGS, the formulated feeds, and the egg yolk lipids. Both R- and L-DDGS had high antioxidant capacities, however, the formulated feeds did not have as pronounced antioxidant capacity as the individual DDGS samples (data not shown), and the diets had no significant impact on the FRAP values of egg yolk (data not shown). Future studies should include identification of water-soluble antioxidant components in DDGS, as well as evaluation of the antioxidant activity of egg yolks using antioxidant assays in which the contribution of lipophilic antioxidants can be adequately assessed.

CONCLUSIONS

Regular and low-fat DDGS addition to layer diets did not influence egg production, egg weight, feed intake, or feed efficiency, confirming earlier studies that 20 to 25% inclusion rate is not detrimental to the performance of the layers. Eggs from layers fed DDGS had enhanced levels of tocopherols, tocotrienols, and xanthophylls in the yolk, which enhanced the yellow and red color of the eggs. Eggs from L-DDGS increased tocopherols more efficiently, but eggs from R-DDGS diets had higher xanthophylls. Thus, inclusion of DDGS in the diet of laying hens resulted in beneficial increases of several beneficial lipophilic nutrients in egg yolks with no apparent detrimental effects.

Based on these findings, future studies will focus on extracted DDGS oil as a feed component and its impact on egg yolk lipid quality and composition.

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SUPPLEMENTARY DATA

Table S1. Analyzed essential amino acid (g/100 g) content of DDGS components and feeds

Table S2a. Main effect treatment differences of R-DDGS and L-DDGS on feed intake and feed efficiency.

Table S2b. Interaction treatment differences of R-DDGS and L-DDGS on egg mass each Time.

Supplementary data is available at PSA *Journal* online.

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